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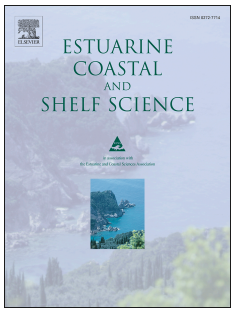

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Ion concentrations in seagrass: A comparison of results from field and controlled-environment studies

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Abstract

Osmoregulation is essential for the survival of seagrasses in marine and hypersaline environments. The aim of this study was to examine ion concentrations of four seagrass species (*Posidonia australis, P. sinuosa, Amphibolis antarctica* and *A. griffithii*) after exposure to salinity changes. Plant fragments were placed in a series of aquaria at marine salinity (35) and, after one week of acclimation, exposed for 7 days to salinities between 20 and 70. Cl^-, Na^+ and total ion concentration increased with salinity in leaf tissue of the four seagrasses species. These results were compared with those of *P. australis* and *A. antarctica* samples collected at three locations at Shark Bay, Western Australia where higher salinities occurred, ranging from 46-51. Concentrations of K^+ and Ca^{+2} were higher in seagrass tissues from Shark Bay than in those in aquarium trials. Cl^-, Na^+ and total ions in *P. australis* and *A. antarctica* from Shark Bay were lowest at the highest salinity location. The K^+/Na^+ ratio in the aquarium trials (under ambient conditions) was in the following order: *A. antarctica = A. grifffithii > P. australis > P. sinuosa* and Ca^{+2}/Na^+ ratio was: *A. antarctica = A. grifffithii > P. sinuosa > P. australis.* This species order indicates a physiological capacity to tolerate variation in salinity. Furthermore, these ratios were higher in the locality with highest salinity in Shark Bay, indicating acclimation and adaptation of ion concentrations to the salinity regime in the environment.

Keywords: ions concentrations; *Posidonia australis; Posidonia sinuosa; Amphibolis antarctica; Amphibolis griffithii*

1. Introduction

1.1. Osmoregulation in seagrasses

Elevated environmental salinities reduce water potential, making it increasingly difficult for plants to acquire water and nutrients for the environment (see Touchette, 2007 for a review). The ability of plants to tolerate salt is determinate by multiple biochemical pathways that facilitate retention and/or acquisition of water, protect chloroplast functions, and maintain ion homeostasis (Parida and Das, 2005). Seagrasses have adapted to life in the sea by developing several strategies to cope with the physiological stresses imposed by salinity (Touchette, 2007), including maintenance of ion homeostasis in order to tolerate salinity changes.

Osmoregulatory adjustments in seagrasses include synthesis of compatible solutes: carbohydrates and free amino acids (Munns, 2002; Touchette 2007), proline and sugars (Tyerman et al., 1984; Pulich, 1986; Koch et al., 2007; Sandoval-Gil et al., 2012, 2014; Marín-Guirao et al., 2013) and changes in cell ultrastructure (Verslues et al., 2006; Sandoval-Gil et al., 2012), but much less is known about changes in ion content (Tyerman et al., 1984; Tyerman, 1989; Marín-Guirao et al., 2013; Garrote Moreno et al., 2014a,b) that enable seagrasses to cope with gradual and/or pulsed changes in seawater salinity.

Recent studies in several species of seagrasses have observed almost a doubling in concentrations of Na^+ and Cl^- at the highest salinities tested under hypersaline conditions (Garrote-Moreno et al., 2014a; Garrote-Moreno et al., 2015). Marine plants must be able to balance Na^+ and Cl^- fluxes to maintain osmotic equilibrium while preventing the accumulation of these toxic ions within the cytoplasm (Touchette, 2007). It is likely that seagrasses growing within their optimal salinity range can achieve equilibrium fairly rapidly. However, plants exposed to waters outside their typical salinity distribution, but within their tolerance range, may require additional time (days to weeks) to acclimate (Tyerman et al., 1984). When salinity changes persist or become more intense, however, the adverse effects of ionic and osmotic stress on metabolism can compromise the productivity and survival of seagrass species (Hasegawa et al., 2000; Zhu, 2003; Parida and Das, 2005; Touchette, 2007). While Cl^- and Na^+ are sequestered in the cell vacuole (Touchette, 2007) they also contribute to maintenance of these ions in low concentration in the cytoplasm preventing physiological damage in addition to osmotic adjustment (Hajibagheri and Flowers, 1989). Maintenance of adequate levels of K^+ and Ca^{+2} is essential for plant survival in counteracting salinity (Maathuis and Amtmann, 1999). Higher K^+/Na^+ and Ca^{+2}/Na^+ ratios are characteristic of more tolerant salinity species (Maathuis and Amtmann, 1999; Muramatsu et al., 2002, Garrote-Moreno et al., 2014a, Garrote-Moreno et al., 2015).

1.2. Seagrasses in hypersaline environments

The extensive and diverse seagrass communities along Western Australia's coastline may be attributed to the general suitability of the coast, which boasts a variety of habitats and a range of tropical and temperate species available for colonization (Kirkman, 1997, Carruthers et al., 2007). *Posidonia australis* and *P. sinuosa* dominate protected habitats in Western Australia, with some *Amphibolis* and other species in small areas. *Amphibolis antarctica* and *P. australis* are Australian endemic seagrasses, widely distributed across southern Australia and reaching their northern tropical limit near Shark Bay on the western coast (Walker, 1985).

Knowledge of salinity thresholds of seagrass species is crucial to understanding and predicting their capacity to withstand chronic changes in salinity regimes, such as hypersaline discharges from desalination plants, and prevent or reduce the impact that this industry may cause some Australian seagrass species. Species of *Posidonia* and *Amphibolis* usually inhabit sublittoral environments with very stable salinity regimes but in Shark Bay (Fig. 1), they also occur in areas with naturally elevated salinity. It is unusual for increased salinity to be maintained at a constant high value in the shallow sedimentary

environments with which seagrasses are associated and in this respect Shark Bay provides an almost unique environment for study. Although there are some variations in surface salinity, bottom salinity remains relatively constant on a seasonal basis (Logan et al., 1974) and on a longer time span, no significant differences in the spatial pattern of salinity distributions were observed by Smith and Atkinson (1983). Shark Bay, Western Australia, consists of two gulfs more than 200 km long; open to the Indian Ocean at their northern ends. The restriction on seawater circulation imposed by broad, shallow shoals across the northern ends of the gulfs combined with the high rate of evaporation in the arid, subtropical climate, results in a gradient in salinity from north to south. Salinity reaches almost double that of seawater salinity at the southern ends of the gulfs, where evaporation exceeds precipitation by a factor of 10, and where there is a persistent gradient in salinity which increases from oceanic (35) to almost twice that of seawater (70) in the southern sections of the bay (Logan et al., 1974; Smith and Atkinson, 1983; Hetzel et al., 2015). Shark Bay has a remarkable and diverse seagrass flora, with 12 species recorded, including some species from the temperate genera, *Posidonia* and *Amphibolis* (Walker et al., 1988). The occurrence of seagrass species is influenced by the prevailing salinity, allowing individual species to be examined along the gradient of salinity at the upper limit of their tolerance to salinity. Extensive seagrass meadows cover much of the vast area of Shark Bay (surface area 13,000 km^2), forming the biggest seagrass banks in the world. They are dominated by *A. antarctica*, which covers 3700 km^2, approximately 85% of the area covered by seagrasses, with smaller areas of *P. australis* (200 km^2) (Walker, 1985). Earlier studies showed a positive correlation in distribution, biomass and in situ productivity of *A. antarctica* with increasing salinity, up to an optimum growth rate at 42, and then decreasing as the salinity increased, as well as at lower oceanic concentrations (Walker, 1985; Walker et al., 1988).

1.3. This study – aims

The aim of this study was to examine changes in concentrations and ratios of Na^+, Ca^{+2}, K^+ and Cl^- in leaf tissue of four SW Australian seagrass species (*P. australis* Hook. *f.*, *P. sinuosa* Cambridge and Kuo, *A. antarctica* (Labill.) Sonder *et* Ascherson and *A. griffithii* den Hartog) over a range of salinities (20-70) in aquarium conditions. These ion concentrations were then compared for two of these species, *P. australis* and *A. antarctica* from three locations in Shark Bay with naturally elevated salinity (Monkey Mia (46), Denham (46.35) and Nanga (51), where the occurrence of seagrass species is influenced by the prevailing salinity.

2. Materials and methods

2.1 Plant sampling and experimental mesocosmos design

Fragments of rhizome with intact connected shoots and roots were collected from a shallow bed at 1-1.5 m depth located at Woodman Point (33^o S, 112^o) near Perth, in October- November 2010. Plants were brought to The University of Western Australia (Perth, Australia) in coolers from the sampling site and transplanted within 4 hours. The mesocosms consisted of six 200 L aquaria in a constant temperature facility at UWA with constant temperature, kept at 22^o C. Increased salinity treatments were produced by

adding marine salt to seawater and the lower salinity was obtained by diluting seawater with distilled water. Salinity levels were maintained within ±1.5 throughout the experiment (here salinity is measured and reported according to the practical salinity scale). The light regime was adjusted to 12:12 h (light:dark) with additional overhead fluorescent lights. Independent air pumps were installed to maintain proper aeration in each aquarium. The parameters of illumination (irradiance and photoperiod), water temperature and water refill were kept constant during all experimental process, so salinity was the only introduced variable after the acclimation period. The species tested have similar thermal optima in the warm temperate range. They inhabit areas where mean water temperatures have a rather restricted annual range (Cambridge et al., 1991; Walker and Cambridge, 1995). After one week acclimation period, plant fragments were exposed for 7 days to 20, 35 (ambient salinity, control treatment), 40, 50, 60 and 70 for *P. australis* and *P. sinuosa*; and 20, 35 (ambient salinity, control treatment), 45, 60 and 70 for *A. antarctica.* Due to the scarcity of *A. griffithii* at the sampling sites, the salinity treatments for this species were restricted to 20, 35 (control treatment) and 70.

P. australis was collected at three locations at Shark Bay some 830 km north of Perth: Monkey Mia (46), Denham (47) and Nanga (51) at approximately 2 m depth. *A. antarctica* was only found at two of the three stations, Monkey Mia and Nanga (Fig. 1). Water samples were measured at each site for analysis of background salinity with a refractometer. The samples were collected by snorkling in the morning and then kept in coolers until processing within a few hours at Denham, using the same techniques as the aquarium samples (described in 2.2).

2.2 Plant response measurements

Total surface area (cm^2 $shoot^{-1}$) and number of leaves per shoot was measured on ten replicates for *P. australis* and *P. sinuosa.*

Leaf ion cations (Na^+, K^+, Ca^{+2}) and anions (Cl^-) concentrations were determined on six samples (replicates) per salinity and species using an ion chromatograph (Metrohm 850 ProfIC AnCat- MCS with chemical suppression and conductimetric detection). Each sample consisted in two leaf pools for *Posidonia* and ten for *Amphibolis*, employing the basal third of photosynthetically developed tissue randomly selected for aquarium or locality on Shark Bay. Each sample of 0.05 g dw of dried (80ºC for 24h) and grounded leaf material, previously cleaned of epiphytes and rinsed with freshwater to remove attached salts (Birch, 1975). The samples were suspended in 15 ml of a 3.5 mM HNO_3 solution made with ultrapure water, stirred for 30 minutes and centrifuged at 5000 rpm for 5 minutes. The supernatant was filtered through 0.45 µm filter and transferred through a Sep-Pak C-18 column to collect the organic matter before it was analyzed in the ion chromatograph (Marín-Guirao et al., 2013).

2.3 Statistical analysis

Leaf ion concentration (for *A. antarctica*) was analyzed using one-way ANOVA with salinity as the single factor. Ions concentration and ratios in leaves of *P. australis* and *P. sinuosa* were analyzed with a two-factor ANOVA with salinity and seagrass species as the factors, followed by Tukey post hoc comparisons. For the two-way ANOVA, Tukey tests comparing salinity treatments were done after pooling the two species for each salinity treatment if the main salinity effect was significant and the interaction term between salinity and seagrass species was not. If the interaction term was significant, one-way ANOVA followed by Tukey tests were done among salinity treatments for each seagrass species separately (Sokal and Rohlf, 1998; Quinn and Keough, 2002). Homogeneity of variance was evaluated with Cochran's test (Underwood, 1997). Differences were considered significant at $p \leq 0.05$.

3. Results

There were significant differences in number of leaves per shoot and total surface between species for *P. australis* and *P. sinuosa* sampled from Woodman Point and between *P. australis* from Shark Bay and Woodman Point (Fig. 2). For *P. australis*, leaf area and number of leaves per shoot in Shark Bay plants were higher than those in aquaria (leaf area 103.86±0.66, 68.63±4.79, number of leaves per shoot 3.33±0.16, 2.68±0.14, respectively).

Mean values of Cl^-, Na^+, and Ca^{+2} measured in *P. sinuosa* control plants were, respectively, 12.6, 17.7 and 27.8% higher than in *P. australis* (Fig.3). Na^+ and Cl^- concentration increased with increased salinity in both seagrasses by the end of the experiment (significant interaction between salinity and seagrass species; Fig.3, Table1). If we compare Cl^- and Na^+ concentration in *P. australis* between control salinity and the highest salinity tested (70) these ions increased 48.5 and 67.6% respectively, and 38.8 and 44.4% for *P. sinuosa*. In contrast, the lowest concentrations of both ions were measured at the highest salinity location in Shark Bay (Monkey Mia > Denham > Nanga).

K^+ concentration at the end of the experiment was lower in the highest salinity treatment (70) than in the rest of treatments for both species (significant interaction between salinity and seagrass species, (Fig.3, Table1). *P. australis* decreased 34.7% at the highest salinity tested compared with control salinity and this decrease was 8.3% for *P. sinuosa*. In contrast to the aquarium trials, the highest concentration of K^+ was found at the highest salinity location in Shark Bay (Nanga > Denham = Monkey Mia).

Ca^{+2} concentration at the end of the experiment increased with increased salinity, and that increase was steeper for *P. australis* (14.4%) than for *P. sinuosa* (1.1%), (significant interaction between salinity and seagrass species; Fig. 3, Table 1). Ca^{+2} concentration for *P. australis* in Shark Bay was lower at the highest salinity location (Nanga < Denham = Monkey Mia) but it was higher than in aquarium conditions.

Total ion concentration at the end of the experiment increased (more than 30%) with increased salinity to a similar extent in both species with no significant interaction between salinity and seagrass species (Fig. 5, Table 1). However, total ion concentration decreased with increased salinity at locations in Shark Bay (Nanga < Denham < Monkey Mia).

The ratio K^+/Na^+ at the end of the experiment decreased with increased salinity, from 0.5 to 0.1 for *P. australis* (61.3%) and for *P. sinuosa* (34.7%), (significant interaction between salinity and seagrass species; Fig. 3, Table 1). There was a small but significant decrease for *P. australis* from the lowest salinity to 50 then a more pronounced decrease from 50 to 70. There was a similar decrease over the plants with *P. sinuosa* but showed a marked decrease at 35. This ratio increased at 40 and 50 and then it decreased greatly (i.e 70 was lower than 35, 35 lower than 20 and 60 , 20 and 60 lower than 40 and 50). In Shark Bay this ratio was higher at the highest salinity (Nanga > Monkey Mia, Nanga = Denham, Denham = Monkey Mia).

Similar results were observed for the Ca^{+2}/Na^+ ratio at the end of the experiment; the ratio decreased with increased salinity, 29.7% for *P. australis* and 26.6% for *P. sinuosa*, with no significant interaction between salinity and seagrass species; (Fig. 3, Table 1). The decrease was gradual, and significant differences were only found between the lowest (20) and the highest salinity treatments (60 and 70). In Shark Bay we observed differences between the lowest salinity location and the other two (Nanga = Denham > Monkey Mia).

Similar results were found for Cl^- and Na^+ concentrations in *A. antarctica* as salinity increased. At the end of the experiment these concentrations increased with increased salinity in a rather gradual manner, 35.7% and 22.7% respectively respect control treatment (Fig. 4, Table 1), with the concentration lowest at 20 and becoming progressively higher at higher salinities. K^+ concentrations in *A. antarctica* at the end of the experiment showed highest value at 60 than at low and high salinities (Fig. 4, Table 1). In Shark Bay Cl^-, Na^+ and Ca^{+2} concentration were higher at the lower salinity location (Monkey Mia > Nanga). A slight decrease with increased salinity (6.7%) was found for Ca^{+2} in *A. antarctica* at the end of the experiment, with 45, 60 and 70 being significantly lower than 20 and 35 (Fig. 4, Table 1). No significant differences were observed between both locations in Shark Bay. Total ion concentration in *A. antarctica* at the end of the experiment increased with increased salinity (21.9%), with 35 and 45 displaying significantly higher concentrations than 20 , 60 and 70 higher values than 35 and 45, (Fig. 5, Table 1). Total ion concentration was higher at the locality with lower salinity (Monkey Mia > Nanga). Both K^+/Na^+ and Ca^{+2}/Na^+ ratios in *A. antarctica* at the end of the experiment decreased with increased salinity (6.1% and 4.7% respectively), (Fig. 4, Table 1). Despite the limited sample size for *A. griffithii*, our results show that, for ambient salinity (35) Cl^-, Na^+, K^+, Ca^{+2} concentration and K^+/Na and Ca^{+2}/Na^+ ratios were similar to *A. antarctica* (Fig. 4, Table 1).

4. Discussion

This study found that Cl^-, Na^+ and total ion concentration increased with salinity (20-70) in leaf tissue of the four seagrasses species in aquarium conditions, similar to other species of seagrass: *P. oceanica* and *Cymodocea nodosa* (Marín-Guirao et al., 2013; Garrote-Moreno et al., 2015), *Thalassia testudinum* and *Halodule wrightii* (Garrote-Moreno et al., 2014b). Suggesting that accumulation of ions is important in

osmotic adjustment. Our plants also followed the same pattern of ion concentration as in these previously reported studies: $Cl^- > Na^+ > K^+ > Ca^{+2}$ for both aquarium and Shark Bay samples.

These ion concentrations were then compared for two of these species, *P. australis* and *A. antarctica* from three locations in Shark Bay with naturally elevated salinity [Monkey Mia (46), Denham (46.35) and Nanga (51)], where the occurrence of seagrass species is influenced by the prevailing salinity. In contrast to the aquarium trials, concentrations of Cl^-, Na^+ and total ions in *P. australis* and *A. antarctica* from Shark Bay were lowest at the highest salinity location (Nanga, 51). K^+ and Ca^{+2} were higher in seagrass tissues from Shark Bay than in those in aquarium trials. The K^+/Na^+ ratio in the aquarium trials (under ambient conditions) was in the following order: *A. antarctica* = *A. grifffithii* > *P. australis* > *P. sinuosa* and Ca^{+2}/Na^+ ratio was: *A. antarctica* = *A. grifffithii* > *P. sinuosa* > *P. australis.* This species order indicates a physiological capacity to tolerate variation in salinity (Maathuis and Amtmann, 1999; Muramatsu et al., 2002; Garrote-Moreno et al. 2014b; Garrote-Moreno et al., 2015). Furthermore, these ratios were higher in the locality with highest salinity in Shark Bay, indicating acclimation of ion concentrations to the salinity regime in the environment. Our results showed a difference in ion ratio, which indicate that *A. antarctica* would have a better salinity tolerance than *P. australis*, and this in fact reflected by their distribution in Shark Bay, where *A. antarctica* penetrates further than *P. australis* into higher salinity waters along the permanent north-south salinity gradient (Walker, 1985; Walker et al. 1988).

The participation of ions in adjusting osmotic potential (Ψ_π) also varies among species. Although Ψ_π or other important measures in osmoregulation was not measured in this study, the clear lineal relationship found between the ionic concentrations and salinity, indicates the involvement of ions in osmotic regulation in these species. In *P. oceanica*, only Cl^- was demonstrated to participate in osmotic adjustment (Marín-Guirao et al., 2013; Garrote-Moreno et al., 2015) while in *C. nodosa* the percentage of ion participation in Ψ_π was clear as it increased in all hypersaline conditions (37-59.5) (Garrote-Moreno et al., 2015).

K^+/Na^+ and Ca^{+2}/Na^+ ratios for *P. australis* were slightly higher for Shark Bay than for the corresponding salinity in the aquaria. However, in aquaria these ratios decreased with increased salinity, whereas in Shark Bay these ratios were higher at the highest salinity location (Nanga, Fig.3). Just as for *P. australis*, *A. antarctica* ratios in Shark Bay for K^+/Na^+ were higher than in aquaria and for Ca^{+2}/Na^+, much higher in Shark Bay (Nanga) than in aquarium conditions (Fig. 4). High salinity competes with the uptake of other ions, especially K^+ (and Ca^{+2}).

The responses of these species under aquarium conditions focused solely on the effects of changes in salinity for adult shoots, after an acclimation period and a week of exposure. Walker and McComb (1990) compared the effect of salinity variations on the growth of *A. antarctica* seedlings, both in situ and in aquaria, and found that its tolerance was similar in both cases. Sensitivity to salinity variations and ions concentration may differ in seedlings (Pujol et al., 2001), different plant tissues (Birch, 1975; Ye and Zhao, 2003; Garrote-Moreno et al., 2014b), time of exposure (minutes to hours to days; Flowers et al., 1977; Tyerman, 1982; Touchette, 2007), exposure and recovery period (Marín-Guirao et al., 2013).

The ion concentrations of *A. antarctica* and *P. australis* collected in Shark Bay are the result of plants fully acclimatized to the prevailing hypersaline conditions (described in Walker, 1985, Walker and McComb, 1990, Hetzel et al., 2015). In contrast, the hypersaline brines discharged by desalination plants present a more complex situation, so that it may not be possible to extrapolate the results obtained in this study to the tolerance of seagrasses exposed to a real brine discharge. However, the constant salinity fluctuations, de-oxygenated water and slightly higher temperatures associated with brines have been shown to cause physiological stress in seagrass species (Garrote-Moreno et al., 2014a).

The differences in ion concentration shown in this work could also be the result of selection for higher tolerance to consistently hypersaline conditions. Differences in genetic structure between Shark Bay and open coast populations exist for *P. australis* (Sinclair et al., 2014), and there are well-documented instances of seagrasses showing differences in behavior between different populations, which have adapted to the local conditions (Benjamin et al., 1999; Kamermans et al., 1999; van Katwijk et al., 1999; Vermaat et al., 2000; Fernández-Torquemada and Sánchez Lizaso, 2011; Sghaier et al., 2012). Most of these differences have consisted of growth and survival responses: for example, Fernández-Torquemada and Sánchez Lizaso (2011) observed greater shoot growth and survival despite salinity variations for both populations and individuals of *Cymodocea nodosa* from the Mar Menor lagoon (42-47) compared to plants from Mediterranean near-shore coast (37-38). On the other hand, Sghaier et al., (2012) observed that the annual leaf production of *C. nodosa* in the Ghar El Melh Lagoon (Tunisia) was approximately ten times higher in a channel with more stable salinity (37-39) than inside the lagoon (37-45). Similarly, Kamermans et al. (1999) and van Katwijk et al. (1999) observed that estuarine *Zostera marina* individuals did not tolerate an increase in salinity as well as individuals from a marine population.

In conclusion, Cl^-, Na^+ and total ion concentration increased with salinity (20-70) in leaf tissue of the four seagrasses species in aquarium conditions. In contrast to the aquarium trials, concentrations of Cl^-, Na^+ and total ions in *P. australis* and *A. antarctica* from Shark Bay were lowest at the highest salinity location (Nanga, 51). K^+ and Ca^{+2} were higher in seagrass tissues from Shark Bay than in those in aquarium trials. The K^+/Na^+ ratio in the aquarium trials (under ambient conditions) was in the following order: *A. antarctica* = *A. grifffithii* > *P. australis* > *P. sinuosa* and Ca^{+2}/Na^+ ratio was: *A. antarctica* = *A. grifffithii* > *P. sinuosa* > *P. australis.* This species order indicates a physiological capacity to tolerate variation in salinity. Furthermore, these ratios were higher in the locality with highest salinity in Shark Bay, indicating acclimation of ion concentrations to the salinity regime in the environment. So, we can conclude that the differences observed in this work between Shark Bay and aquaria conditions were due to the adaptation of different population of the same species to their habitats and an ion acclimation of these species with their environment.

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Fig. 1. Situation of the three localities where samples were collected in Shark Bay (Western Australia).

Fig. 2. Mean (±SE) number of leaves per shoots and total surface in aquarium conditions and Shark Bay for *P. australis* and number of leaves per shoots and total surface in aquarium conditions for *P. sinuosa*.

Fig. 3. The effects of salinity on leaf ion concentration (mg g^{-1}) and K^+/Na and Ca^{+2}/Na^+ ratios in aquarium conditions and Shark Bay for *P. australis* and *P. sinuosa*. Solid circles correspond to *P. australis*, open circles to *P. sinuosa* and gray triangles to *P. asutralis* in the three location sampled in Shak Bay [Monkey Mia (46), Denham (46.35) and Nanga (51)]. Values are to means values and confidence intervals are S.E. Letters denote the results of post hoc Tukey comparisons (one-way ANOVA or two-way ANOVA with non-significant interaction term). Insets of ordered salinity treatments denote the results of post hoc Tukey comparisons for each species (two-way ANOVA with significant interaction term). See text for details.

Fig.4. The effects of salinity on leaf ion concentration (mg g^{-1}) and K^+/Na and Ca^{+2}/Na^+ ratios in aquarium conditions and Shark Bay for *A. antarctica* and *A. griffithii*. Diamond circles correspond to *A. antarctica*, open diamonds to *A. griffithii* and gray triangles to *A. antarctica* in the two location sampled in Shak Bay [Monkey Mia (46) and Nanga (51)]. Values are to means values and confidence intervals are S.E. Letters denote the results of post hoc Tukey comparisons (one-way ANOVA).). Due to the reduce samples side of *A. griffithii* only some of the salinity treatments existed. Such values are plotted for illustrative purposes. See text for details.

Fig.5. The effects of salinity on total ion concentration (mg g−1) in aquarium conditions and Shark Bay. (A) Solid circles correspond to *P. australis*, open circles to *P. sinuosa* and gray triangles to *P. australis* in the three location sampled in Shak Bay [Monkey Mia (46), Denham (46.35) and Nanga (51)]. (B) Diamond circles correspond to *A. antarctica*, open diamonds to *A. griffithii* and gray triangles to *A. antarctica* in the two location sampled in Shak Bay [Monkey Mia (46) and Nanga (51)]. Values are to means values and confidence intervals are S.E. Letters denote the results of post hoc Tukey comparisons (one-way ANOVA or two-way ANOVA with non-significant interaction term). Due to the reduce samples side of *A. griffithii* only some of the salinity treatments existed. Such values are plotted for illustrative purposes. See text for details.

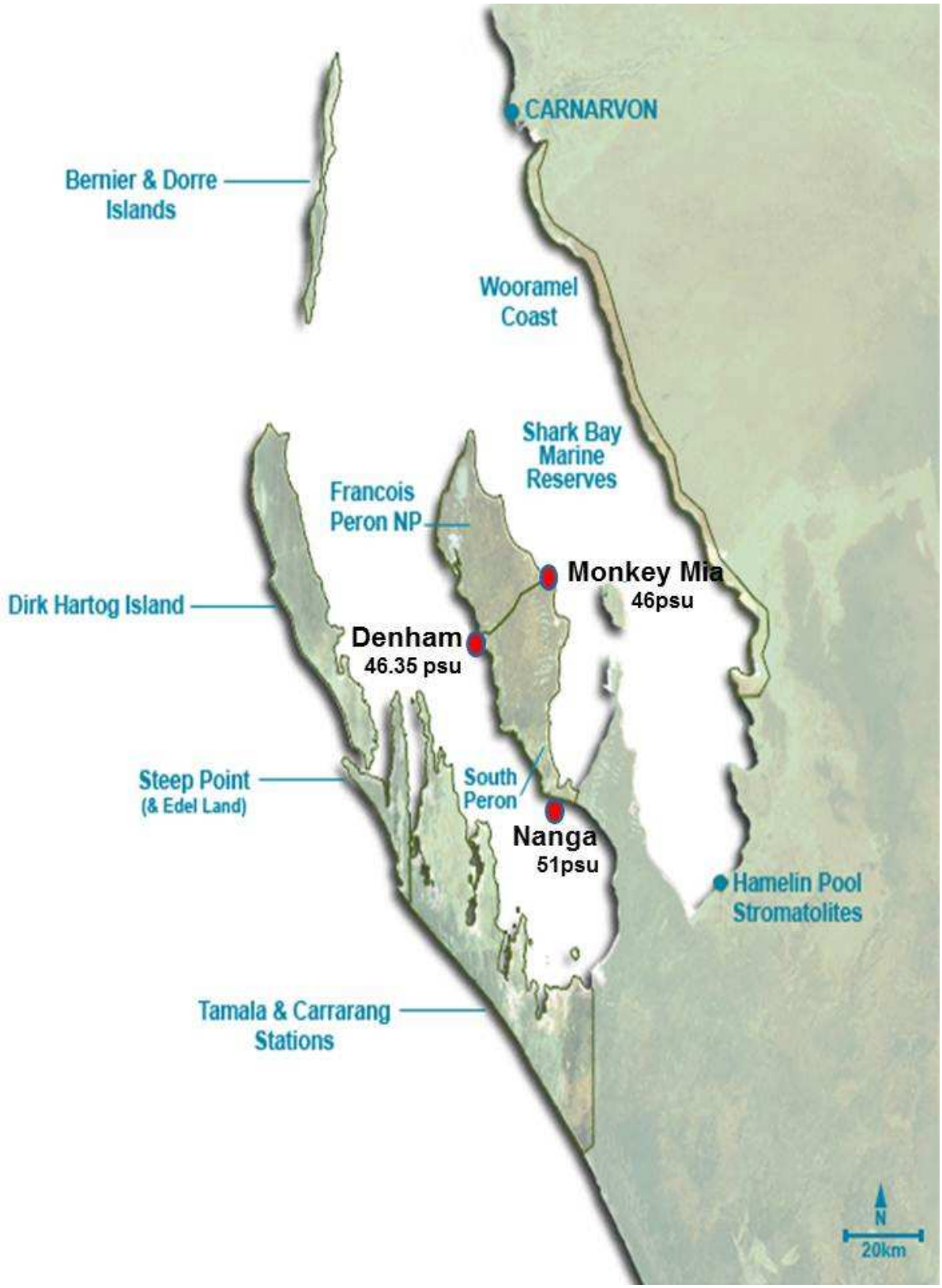
Table 1

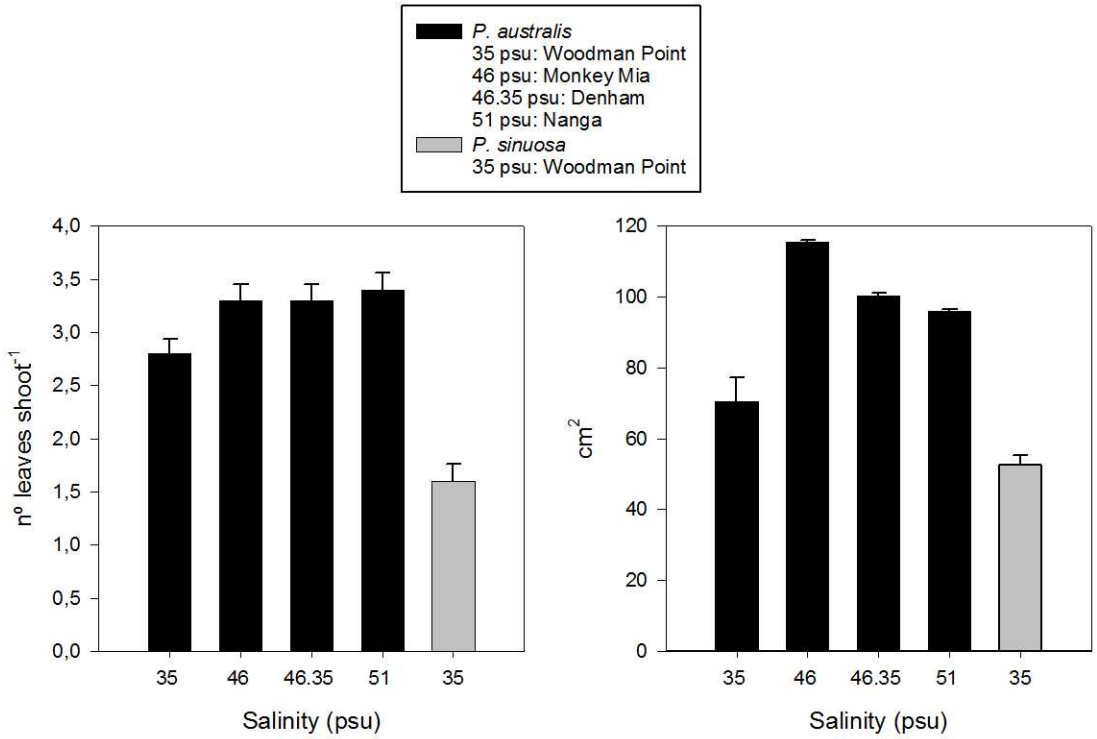
ANOVA summary table comparing variations in ion concentrations in leaves for *P. australis*, *P. sinuosa* and *A. antarctica*.

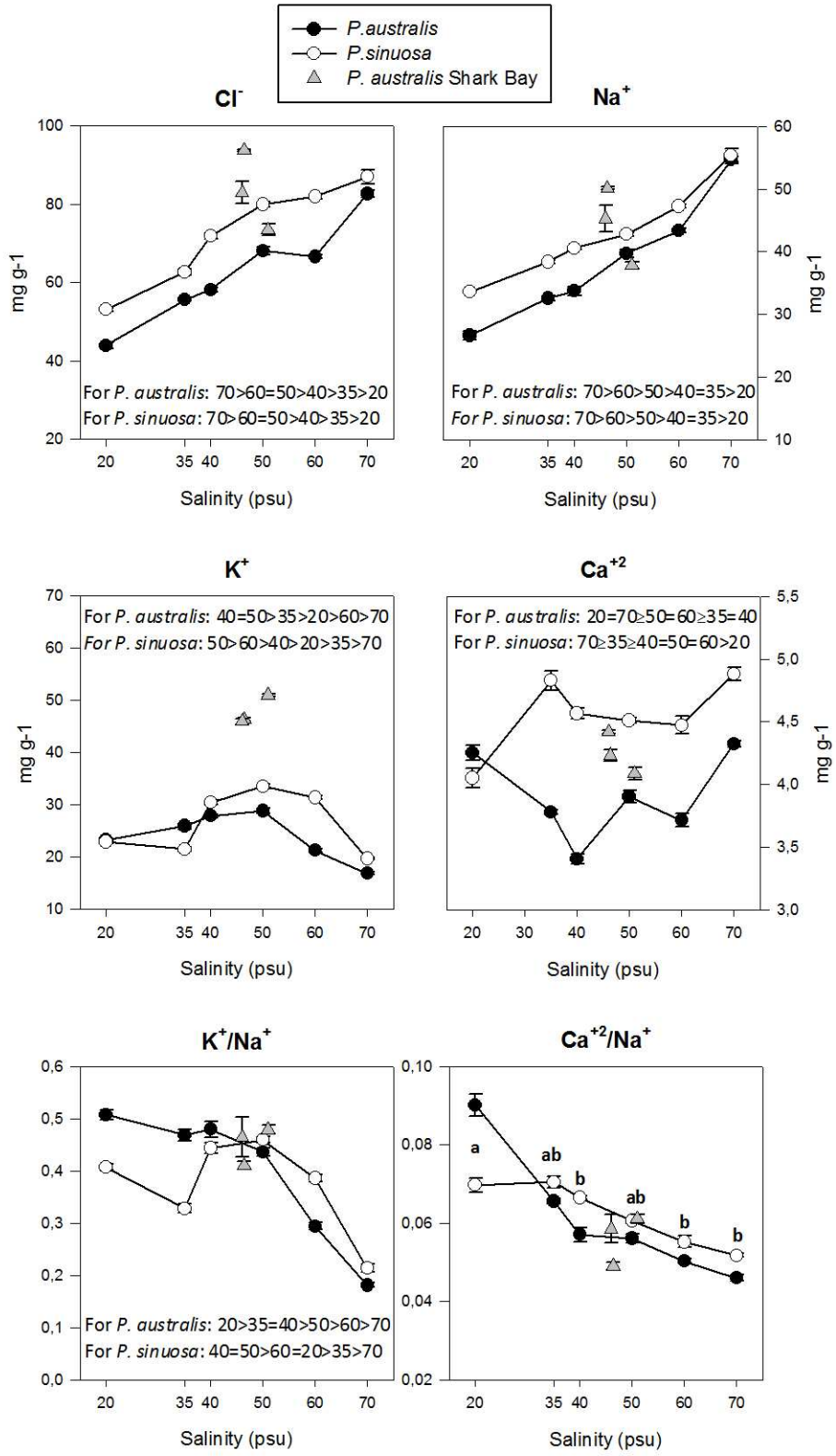
Variable	Effect	df	MS	F
Cl^-	Sal	5	0.18	459.73***
	Species	1	246.70	632541.17***
	SalxSpecies	5	0.10	252.88***
	Residual	60	0.00	
	Total	71		
Na^+	Sal	5	5.37	470.56***
	Species	1	2.58	225.90***
	SalxSpecies	5	0.13	11.57***
	Residual	60	0.01	
	Total	71		
K^+	Sal	5	249.42	301.32***
	Species	1	119.02	143.79***
	SalxSpecies	5	69.33	83.75***
	Residual	60	0.83	
	Total	71		
Ca^{+2}	Sal	5	1.97	4.04**
	Species	1	19.92	40.91***
	SalxSpecies	5	2.51	5.15***
	Residual	60	0.49	
	Total	71		
Total	Sal	5	8.84	17.33***
	Species	1	42.81	83.90***
	SalxSpecies	5	0.36	0.71ns

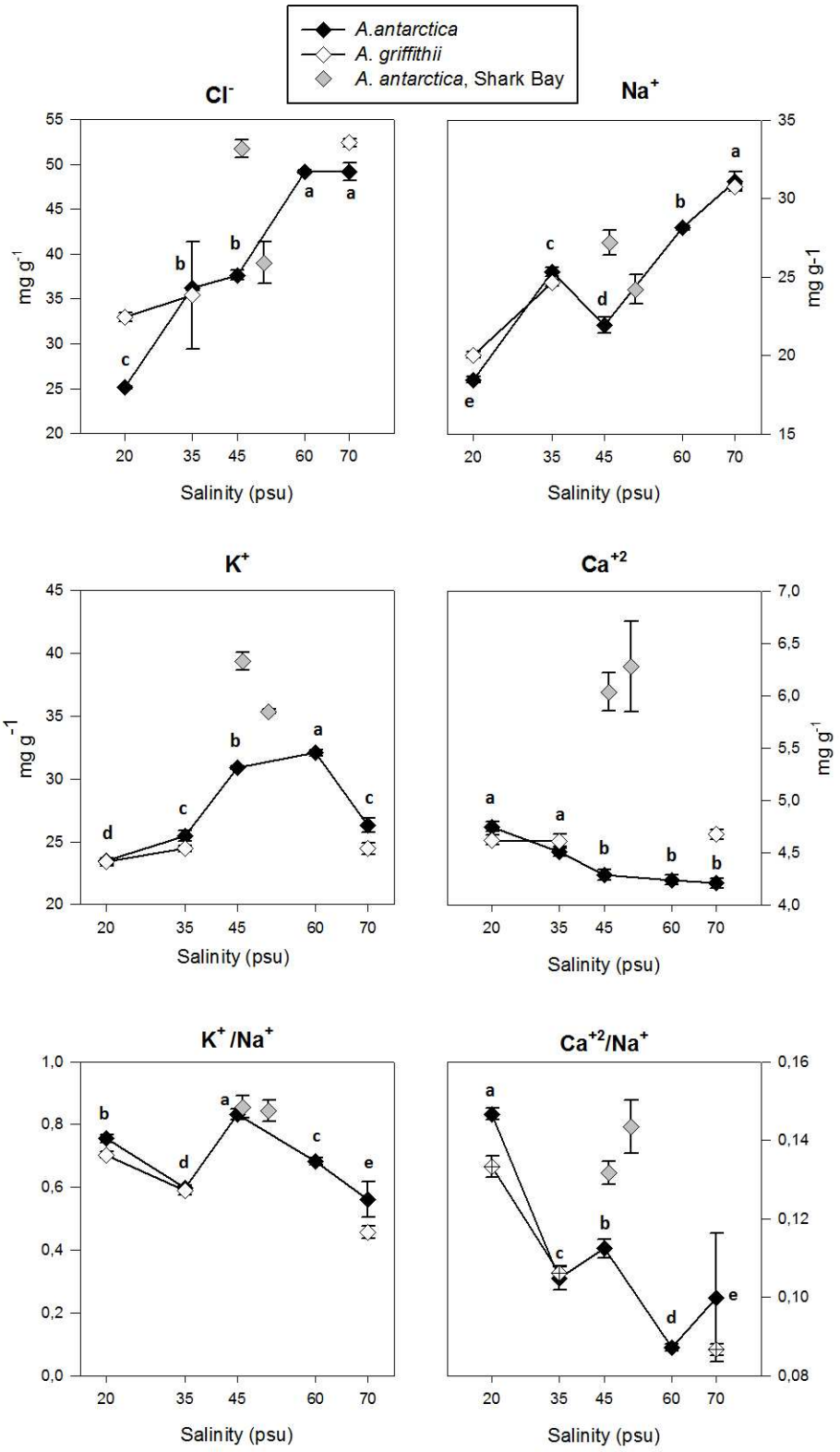
	Residual	60	0.51	
	Total	71		
K^+/Na^+	Sal	5	0.12	263.41***
	Species	1	0.01	19.96***
	SalxSpecies	5	0.02	52.55***
	Residual	60	0.00	
	Total	71		
Ca^{+2}/Na^+	Sal	5	0.00	3.97**
	Species	1	0.00	7.05*
	SalxSpecies	5	0.00	1.96ns
	Residual	60	0.00	
	Total	71		
A. antarctica				
Cl-	Sal	4	629.41	229.18***
	Residual	25	2.75	
	Total	29		
Na^+	Sal	4	0.27	136.17***
	Residual	25	0.00	
	Total	29		
K^+	Sal	4	82.71	97.81***
	Residual	25	0.85	
	Total	29		
Ca^{+2}	Sal	4	0.38	17.94***
	Residual	25	0.02	
	Total	29		
Total	Sal	4	4.51	243.50***
	Residual	25	0.02	
	Total	29		
K^+/Na^+	Sal	4	0.11	84.88***
	Residual	25	0.00	
	Total	29		
Ca^{+2}/Na^+	Sal	4	0.00	203.46***
	Residual	25	0.00	
	Total	29		

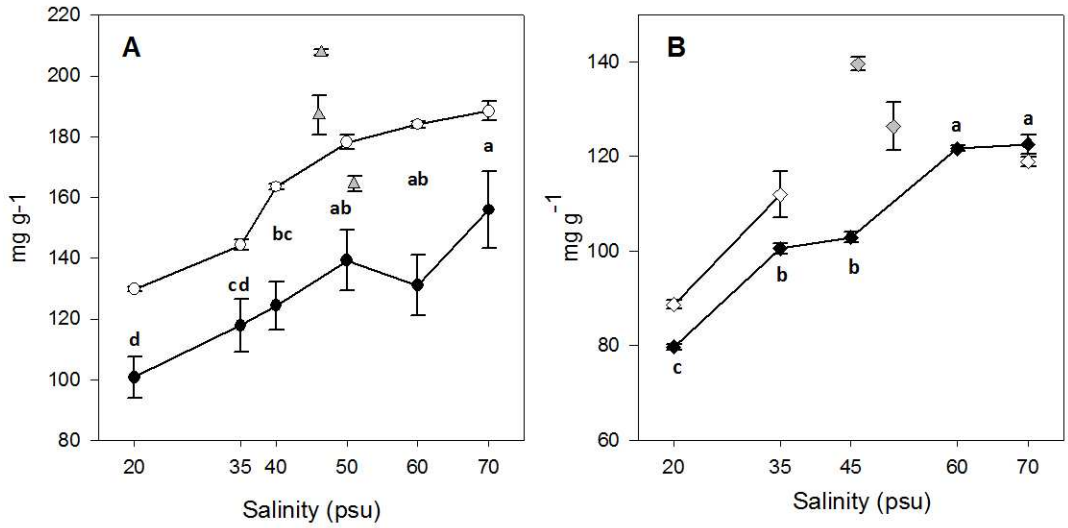
Df, degrees of freedom; MS, mean squares.
* Significant at $p < 0.05$.
** Significant at $p < 0.01$.
*** Significant at $p < 0.001$.


CARNARVON
Bernier & Dorre Islands
Wooramel Coast
Shark Bay Marine Reserves
Francois Peron NP
Monkey Mia
46psu
Dirk Hartog Island
Denham
46.35 psu
Steep Point
(& Edel Land)
South Peron
Nanga
51psu
Hamelin Pool Stromatolites
Tamala & Carrarang Stations
N
20km


P. australis
35 psu: Woodman Point
46 psu: Monkey Mia
46.35 psu: Denham
51 psu: Nanga
P. sinuosa
35 psu: Woodman Point
nº leaves shoot-1
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
35
46
46.35
51
35
Salinity (psu)
cm2
120
100
80
60
40
20
0
35
46
46.35
51
35
Salinity (psu)


P.australis
P.sinuosa
P. australis Shark Bay
Cl-
Na+
K+
Ca+2
K+/Na+
Ca+2/Na+
mg g-1
Salinity (psu)
For P. australis: 70>60=50>40>35>20
For P. sinuosa: 70>60=50>40>35>20
For P. australis: 70>60>50>40=35>20
For P. sinuosa: 70>60>50>40=35>20
For P. australis: 40=50>35>20>60>70
For P. sinuosa: 50>60>40>20>35>70
For P. australis: 20=70≥50=60≥35=40
For P. sinuosa: 70≥35≥40=50=60>20
For P. australis: 20>35=40>50>60>70
For P. sinuosa: 40=50>60=20>35>70
a
ab
b
ab
b
b


A.antarctica
A. griffithii
A. antarctica, Shark Bay
Cl-
Na+
K+
Ca+2
K+ /Na+
Ca+2/Na+
mg g-1
Salinity (psu)
20
35
45
60
70


A
mg g-1
220
200
180
160
140
120
100
80
20
35
40
50
60
70
Salinity (psu)
d
cd
bc
ab
ab
a
B
mg g-1
140
120
100
80
60
20
35
45
60
70
Salinity (psu)
c
b
b
a
a

Highlights

Cl^-, Na^+ and total ion concentration increased with salinity in leaf tissue of *A. antarctica, A. grifffithii*, *P. australis* and *P. sinuosa*.

K^+ and Ca^{+2} were higher in seagrass tissues from Shark Bay than in those in aquarium trials.

Cl^-, Na^+ and total ions in *P. australis* and *A. antarctica* from Shark Bay were lowest at the highest salinity location.

The K^+/Na^+ ratio in the aquarium trials (under ambient conditions) was: *A. antarctica* = *A. grifffithii* > *P. australis* > *P. sinuosa*.

The Ca^{+2}/Na^+ ratio was: *A. antarctica* = *A. grifffithii* > *P. sinuosa* > *P. australis.*